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Identification of F-box proteins that are involved in resistance to methylmercury in *Saccharomyces cerevisiae*

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Abstract **We searched for F-box proteins that might be related to the mechanism that protects *Saccharomyces cerevisiae* against the toxic effects of methylmercury. We found that overexpression of Hrt3 and of Ylr224w rendered yeast cells resistant to methylmercury. Yeast cells that overexpressed Hrt3 and Ylr224w were barely resistant to methylmercury in the presence of a proteasome inhibitor. Our results suggest the existence of some protein(s) that enhances the toxicity of methylmercury in yeast cells and, also, that overexpression of Hrt3 or Ylr224w can confer resistance to methylmercury by enhancing the polyubiquitination of this protein(s) and its degradation in proteasomes.**

Keywords: Methylmercury; Resistance; Ubiquitin; F-box protein; Yeast

1. Introduction

Methylmercury is concentrated in fish via the food chain and, thus, most of the methylmercury that humans ingest comes from fish. Ingestion of methylmercury can cause severe damage to the human central nervous system [1,2]. However, mechanisms of methylmercury toxicity and cellular protective mechanisms against such toxicity remain poorly understood.

In order to characterize cellular mechanisms that protect against methylmercury toxicity, we have been searching for genes involved in the resistance to methylmercury in the budding yeast *Saccharomyces cerevisiae*, a unicellular eukaryote, many of whose gene products have functions similar to those of mammalians, including *Homo sapiens*. As a result, we have identified genes designated *BOP3* [3], *CDC34* [4] and *GFAT* [5], among others. The *CDC34* gene encodes Cdc34, a ubiquitin-conjugating enzyme [6] that is involved in the ubiquitin–proteasome system (UP system). The UP system is a proteolytic pathway that is strongly conserved in eukaryotes and operates as follows. A protein becomes bound to ubiquitin in the cell as a consequence of the actions of three enzymes, namely, a ubiquitin-activating enzyme (designated, generically, E1), a ubiquitin-conjugating enzyme (E2) and a ubiquitin ligase (E3). The resultant polyubiquitinated protein is then recognized and degraded by the proteasome [7–9]. We have analyzed the mechanism of acquisition of resistance to methylmercury that involves the overexpression of Cdc34 and we have shown that enhancement of cellular proteolysis by the UP system helps to protect cells against the toxic effects of methylmercury [10]. Thus, we postulated that some protein(s) involved in methylmercury toxicity might be included among the proteins whose degradation is enhanced by the enhanced activity of the UP system. Identification of such a protein(s) would provide an important clue to the mechanism of expression of the toxicity of methylmercury.

The SCF (*S*kp1/*C*dc53/*F*-box protein) complex is known as an E3 that is involved in the polyubiquitination of proteins in cooperation with Cdc34 (E2) [11,12]. Among the factors that make up this SCF complex in budding yeast, 17 different F-box proteins are known to bind directly to substrate proteins that are then degraded by the UP system [13–16]. F-box proteins have their own respective substrate-specificities, playing important roles in the selection of proteins that are degraded by the UP system. In order to identify the protein(s) involved in methylmercury toxicity that is degraded by the UP system, we need to identify the F-box protein(s) that is involved in the recognition of this protein(s). In the present study, therefore, we searched for F-box proteins that might be involved in the protection of yeast cells against the methylmercury toxicity.

2. Materials and methods

2.1. Culture and transformation of yeast cells

Saccharomyces cerevisiae W303B (*MAT*α *his3 can1-100 ade2 leu2 trp1 ura3*) was grown at 30 °C in yeast extract–peptone–dextrose (YPD) medium or synthetic dextrose (SD) medium. Plasmid DNA was introduced into W303B cells by the high-efficiency lithium acetate transformation method [17].

2.2. Construction of plasmids

The genes for F-box proteins were amplified by the polymerase chain reaction (PCR) with yeast genomic DNA as template and the following oligonucleotides as primers: CDC4-F and CDC4-R for the *CDC4* gene; COS3-F and COS3-R for the *COS3* gene; CTF13-F and CTF13-R for the *CTF13* gene; DIA2-F and DIA2-R for the *DIA2* gene; ELA1-F and ELA1-R for the *ELA1* gene; FLM13-F and FLM13-R for the *FLM13* gene; GRR1-F and GRR1-R for the *GRR1* gene; HRT3-F and HRT3-R for the *HRT3* gene; MET30-F and MET30-R for the *MET30* gene; RCY1-F and RCY1-R for the

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RCY1 gene; UFO1-F and UFO1-R for the *UFO1* gene; YDR131C-F and YDR131C-R for the *YDR131C* gene; YDR219C-F and YDR219C-R for the *YDR219C* gene; YDR306C-F and YDR306C-R for the *YDR306C* gene; YJL149W-F and YJL149W-R for the *YJL149W* gene; YLR224W-F and YLR224W-R for the *YLR224W* gene; YNL311C-F and YNL311C-R for the *YNL311C* gene; FLAG-HRT3-F and HRT3-R for the FLAG-*HRT3* gene; FLAG-YLR224W-F and YLR224W-R for the FLAG-*YLR224W* gene; and SKP1-F and HA-SKP1-R for the *SKP1*-HA gene (see Table 1 for the sequences of all primers). The PCR-generated *CTF13*, *ELA1*, *UFO1*, *YDR219C* and *YJL149W* genes were ligated into the pGEM-T Easy vector (Promega, Madison, WI). Each insert was digested with the restriction endonuclease *Eco*RI and fragments were ligated into the pKT10-GAPDH (*URA3*) yeast expression vector. The PCR-generated *DIA2*, *FLM13*, *HRT3*, FLAG-*HRT3*, *RCY1*, *YDR131C*, *YDR306C* and *YNL311C* genes were ligated into the pTARGET vector (Promega). Each insert was digested with restriction endonucleases, as follows: *Sal*I and *Xho*I for the *DIA2* gene; and *Bam*HI and *Kpn*I for the *FLM13* and *RCY1* genes; and *Kpn*I and *Xho*I for the *HRT3*, *HRT3*-FLAG, *YDR131C*, *YDR306C* and *YNL311C* genes. The resultant fragments were ligated into the pKT10-GAPDH (*URA3*) yeast expression vector. The PCR-generated *CDC4*, *COS3*, *GRR1*, *MET30*, *YLR224W* and FLAG-*YLR224W* genes were ligated into the blunted *Puv*II cloning site of the pKT10-GAPDH (*URA3*) yeast expression vector. The PCR-generated *SKP1*-HA gene was ligated into the blunted *Puv*II cloning site of the pKT10-GAPDH (*TRP1*) yeast expression vector. Sequences of constructs were verified with an automated sequencer.

Table 1
Oligonucleotide primers used in this study

Primer	Oligonucleotide (5′ → 3′)
CDC4-F	GGCAAAAATTACGCTGTACG
CDC4-R	TGCTTATTCTCTCTGGGAAAGG
COS3-F	CTCGAAGCAAGAGGGGAAAAG
COS3-R	TGCTGTTAAAAGAGAGCAGGC
CTF13-F	TGACTGTGAGTCCCCAGAAGT
CTF13-R	TAAAATACCGCCGGTTTTCC
DIA2-F	GACATGCAAAATGATTAGCC
DIA2-R	AGGATACTGCATTATCATCAG
ELA1-F	AAATCGATTGATGTCGAGAT
ELA1-R	GCCTTCGGAGTTGGGTTACT
FLM13-F	TTAGTTACTAAAAGGCTCACA
FLM13-R	TGCTACTTTTGGAAACCTCC
GRR1-F	GTTTTGCGGTTTCCTTTATAC
GRR1-R	GGACAGTAAGTATTCAATGA
HRT3-F	CCATAAGCTAAACTCAAGG
HRT3-R	AACAACTGCAAAAAACATCG
MET30-F	GGGTGTGTGTTTGGTGATTTA
MET30-R	CAAGAAAAGACCACACACAGG
RCY1-F	AAACCAAAAGAAAACAAAAGC
RCY1-R	TCCGCACTTCATACCTAT
UFO1-F	CCGACACTAGGGAATAAGACA
UFO1-R	TGCTCTTCCAAATGTACATAC
YDR131C-F	TTTGAAAGGGCCCGAAAA
YDR131C-R	TAACCGCCATGTCTCACAGTA
YDR219C-F	ATAGTTCCTTCAACCACATAG
YDR219C-R	AAAGTCGGTTTGAGGCGTTT
YDR306C-F	CATATCAACCACAGTACTCAG
YDR306C-R	CACTGACTCTTATAAAACAAA
YJL149W-F	CACAGTGTTTACAACTCAGC
YJL149W-R	TATTTGAAGGGGAGTTGA
YLR224W-F	ATTGGCGCAAAGAAGACAGA
YLR224W-R	GCATAGACGTATATACACAT
YNL311C-F	ACGTTCAAACCAACCGAATC
YNL311C-R	AAAGTCCACTACAAAAAGTCA
FLAG-HRT3-F	AACTCAAGGAGCAAATGGACTACAAGGATGACG ATGACAAGATAGTAGATTATGAAAA
FLAG-YLR224W-F	AGAGATGGACTACAAGGATGACGATGACAAGAA TCAGAGCGATAGCAGCT
SKP1-F	CTAACAACGTAGCGCAGAT
HA-SKP1-R	TAGGCTAAGCGTAATCTGGAACATCGTATGGGTA ACGGTCTTCAGCCCATTC
HRT3ΔF-F	GTGCCATTTAAAGGTACCGCGAAGTACATATATTC
HRT3ΔF-R	CGTCAGGCAAGATGGTACCAATCCAGCAGGGTTG
YLR224WΔF-F	CCACAGCTATAAGGGTACCAGTTTGGCGTGG
YLR224WΔF-R	CCAGTGGTAAATCGGTACCGCTGCTATCGCTC

2.3. Quantitation of the toxicity of methylmercury in yeast cells

Yeast cells (10^4 cells/200 μl) were grown in SD (−Ura) liquid medium that contained methylmercuric chloride at various concentrations. After incubation for 48 h, we measured the absorbance of each culture at 600 nm to quantify cell growth. For the colony-formation assay, we cultured yeast cells (10^6 cells/ml) in SD (−Ura) liquid medium that contained methylmercuric chloride (1 μM). After incubation for 3 h at 30 °C, cells were pelleted by centrifugations and each pellet was suspended and diluted in 0.1 ml sterilized water to yield 10^7, 10^6 and 10^5 cells/ml. Five microliters of each suspension of yeast cells were spotted on agar-solidified SD (−Ura) medium and formation of colonies was examined after culture for 48 h at 30 °C. Yeast cells transformed with the plasmid pKT10-GAPDH were used as controls.

2.4. Site-directed mutagenesis

Site-directed mutagenesis of the genes for Hrt3 or Ylr224w was performed, as described elsewhere [18], with a kit for site-directed mutagenesis from Stratagene (Cedar Creek, TX) according to the manufacturer's instructions. We constructed the FLAG-*HRT3ΔF* and FLAG-*YLR224WΔF* genes by creating pairs of *Kpn*I sites in the open reading frames (ORFs) of the FLAG-*HRT3* or FLAG-*YLR224W* genes and excising the fragments between the respective pairs of *Kpn*I sites. We amplified fragments by PCR using plasmids pKT10-FLAG-*HRT3* and pKT10-FLAG-*YIL224W* as templates and the following oligonucleotides as primers: HRT3ΔF-F and HRT3ΔF-R for deletion of the F-box domain of Hrt3; and YLR224WΔF-F and YLR224WΔF-R for deletion of the F-box domain of Ylr224w. After creation of each pair of *Kpn*I sites, the plasmid was cleaved with *Kpn*I and self-ligated. All mutations were confirmed by DNA sequencing. The resultant plasmids were designated pKT10-FLAG-*HRT3ΔF* and pKT10-FLAG-*YLR224W ΔF*.

2.5. Immunoprecipitation and immunoblotting

Immunoprecipitation and immunoblotting were performed as described elsewhere [19]. Yeast cells (10^7 cells/ml) were grown in 40 ml of SD (−Ura, −Trp) liquid medium for 5 h and harvested. Approximately 0.1 ml of cell pellet was suspended in 0.4–0.5 ml of buffer C [20 mM Tris–HCl (pH 7.5), 1 mM EDTA, 5 mM $MgCl_2$, 50 mM KCl, 5% glycerol, 3 mM DTT, 1 mM phenylmethylsulfonyl fluoride, and 1 μg/ml pepstatin A] and lysed with glass beads (425–600 μm; Sigma, St. Louis, MO), in a cell disruptor (Micro Smash™ MS-100R; Tomy, Tokyo, Japan). Cell extracts were adjusted to equal volumes and concentrations of protein, and analyzed either by immunoprecipitation, or directly by immunoblotting. The Skp1-HA protein was immunoprecipitated from cell extracts (1 mg of protein) using the monoclonal HA-specific affinity matrix clone 3F10 (Roche, Indianapolis, IN). After incubation with cell extract overnight at 4 °C, beads were washed five times with Tris-buffered saline [20 mM Tris–HCl (pH 7.4), 500 mM NaCl] and then the protein was eluted by boiling in sample buffer for SDS–PAGE. Eluates and cell extracts (20 μg of protein) were fractionated by SDS–PAGE (12.5%), and then bands of protein were transferred to an Immobilon-P membrane (Millipore, Bedford, MA). FLAG-Hrt3 and FLAG-Ylr224w were visualized with FLAG-specific monoclonal antibodies (Sigma) and peroxidase-conjugated goat antibodies against mouse immunoglobulins (Dako A/S, Glostrup, Denmark) as primary and secondary antibodies, respectively.

3. Results

3.1. Search for F-box proteins involved in protection of yeast cells against methylmercury toxicity

We generated 17 yeast strains that overexpressed each of 17 different proteins, which have F-box domain, in an attempt to identify the F-box proteins involved in protection of yeast cells

against methylmercury toxicity, and we examined the sensitivity of each of these strains to methylmercury by monitoring colony formation on agar-solidified medium and rates of cell proliferation in liquid medium. We treated yeast cells that overexpressed each F-box protein with 1 μM methylmercury for the colony-formation assay, and we found that the sensitivity of yeast cells that overexpressed Cos111, Ctf13, Dia2, Flm1, Met30, Ydr219c, Ydr306c or Yj1149w was similar to that of control cells (Fig. 1A). By contrast, yeast cells that overexpressed Cdc4, Ela1, Rcy1 or Ynl311c were somewhat

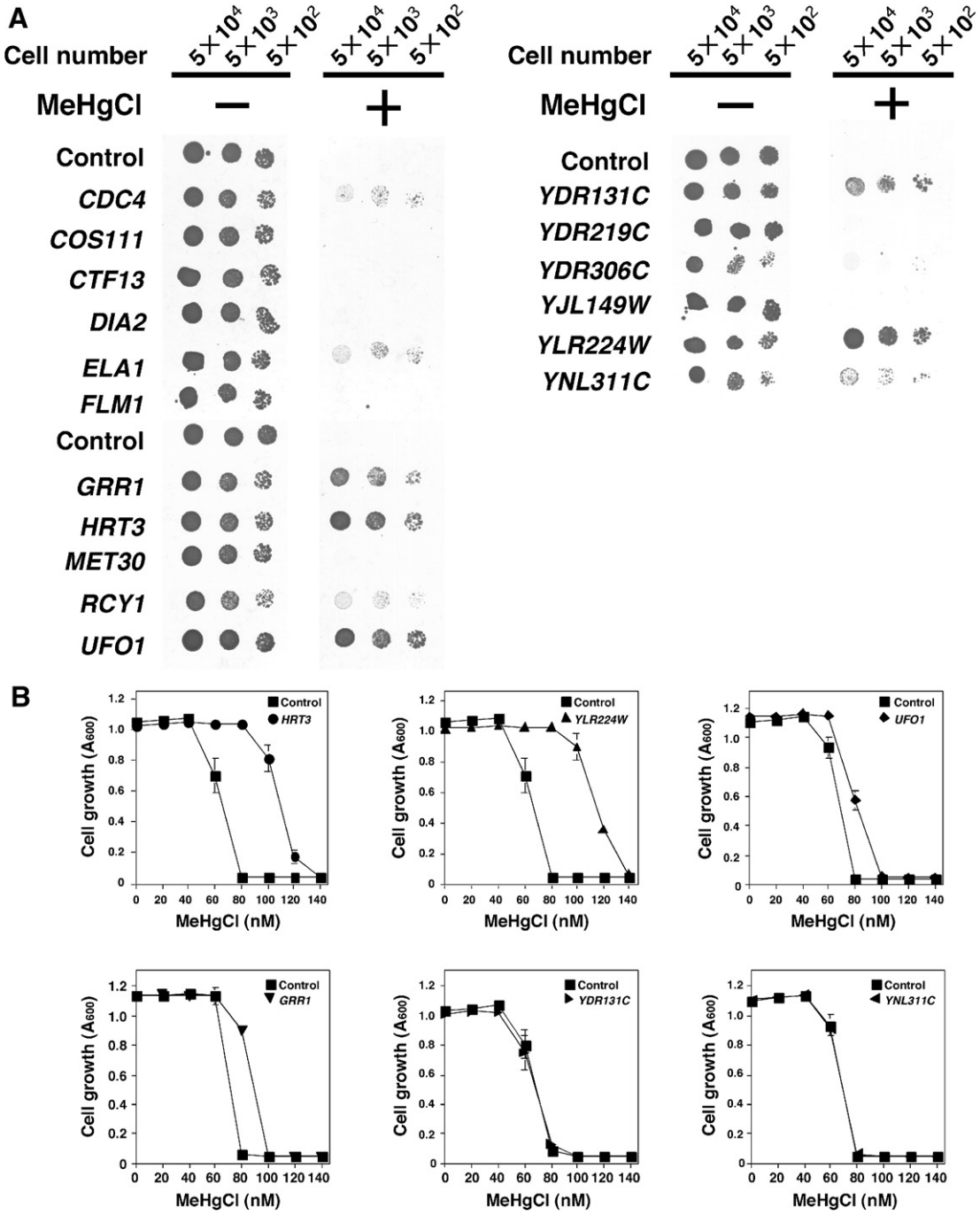

Fig. 1. Sensitivity to methylmercury (MeHgCl) of yeast cells that overexpressed genes for various F-box proteins. (A) Yeast cells (10^6 cells/ml) that harbored pKT10, pKT10-*CDC4*, pKT10-*COS111*, pKT10-*CTF13*, pKT10-*DIA2*, pKT10-*ELA1*, pKT10-*FLM1*, pKT10-*GRR1*, pKT10-*HRT3*, pKT10-*MET30*, pKT10-*RCY1*, pKT10-*UFO1*, pKT10-*YDR131C*, pKT10-*YDR219C*, pKT10-*YDR306C*, pKT10-*YJL149W*, pKT10-*YLR224W* or pKT10-*YNL311C* were grown in SD (−Ura) liquid medium with or without methylmercury (1 μM). After incubation for 3 h at 30 °C, cells of each strain were diluted in sterilized water to 10^7, 10^6 and 10^5 cells/ml. Five microliters of each resultant suspension of cells were spotted on agar-solidified SD (−Ura) medium. Plates were photographed after incubation for 48 h at 30 °C. Three separate experiments were performed and the results were reproducible. (B) Yeast cells (10^4 cells/200 μl/well) that harbored pKT10, pKT10-*HRT3*, pKT10-*YLR224W*, pKT10-*UFO1*, pKT10-*GRR1*, pKT10-*YDR131C* or pKT10-*YNL311C* were grown in SD (−Ura) liquid medium that contained methylmercury at the indicated concentration. After incubation for 48 h at 30 °C, absorbance was measured spectrophotometrically at 600 nm. Each point represents the mean value of results from three cultures with SD (bars). The absence of a bar indicates that the SD falls within the symbol.

resistant to methylmercury (Fig. 1A). However, yeast cells that overexpressed Grr1, Hrt3, Ufo1, Ydr131c or Ylr224w were strongly resistant to methylmercury, as compared with the control cells (Fig. 1B). We next examined the sensitivity of the 17 yeast strains to methylmercury monitoring by growth rates in liquid medium. Only yeast cells that overexpressed Grr1, Hrt3, Ufo1 or Ylr224w exhibited clear resistance to methylmercury, as compared with the control cells, and the cells that overexpressed Hrt3 or Ylr224w were particularly resistance to methylmercury (Fig. 1B). These results suggested that Cdc4, Ela1, Grr1, Hrt3, Rcy1, Ufo1, Ydr131c, Ylr224w and Ynl311c might be F-box proteins that are involved in protection against methylmercury and that Hrt3 and Ylr224w, in particular, might play a major role in such protection. The yeast cells that overexpressed Ydr131c or Ynl311c were not resistant to methylmercury in liquid medium, perhaps because proteins that were expressed at high levels only when yeast cells were cultured on agar-solidified medium might have been involved in the protective mechanism. Therefore, we focused, in the present study, on Hrt3 and Ylr224w, both of which rendered yeast cells strongly resistant to methylmercury.

3.2. Characterization of Hrt3 and Ylr224w

It has been proposed that F-box proteins bind to Skp1, a constituent of the E3 complex, via their respective F-box domains to form an SCF complex (E3) [20,21]. Both Hrt3 and Ylr224w have an F-box domain and both were identified in this study as proteins involved in protection against methylmercury toxicity. However, their functions as F-box proteins have not yet been confirmed. Therefore, we examined whether formation of an SCF complex is necessary for acquisition of resistance to methylmercury when Hrt3 and Ylr224w, respectively, are overexpressed in yeast cells. First, we expressed fusion proteins in which a FLAG tag was fused to the amino terminus of each protein (FLAG-Hrt3 and FLAG-Ylr224w) and proteins in which a FLAG tag was fused to the amino terminus of derivatives of Hrt3 and Ylr224w with deleted F-box domains (FLAG-Hrt3ΔF and FLAG-Ylr224wΔF) in yeast cells in which we also expressed Skp1 fused to an HA tag at its carboxyl terminus (Skp1-HA). We immunoprecipitated extracts of these cells with HA-specific antibodies and then immunoblotted the immunoprecipitates with anti-FLAG-specific antibodies. We confirmed that wild-type FLAG-Hrt3 and FLAG-Ylr224w bound to Skp1-HA, while binding of the derivatives with deleted F-box domains (FLAG-Hrt3ΔF and FLAG-Ylr224wΔF) and Skp1-HA was undetectable (Figs. 2A and B). These results are the first, to our knowledge, to demonstrate the binding of Hrt3 and Ylr224w to Skp1 through their F-box domains and they suggest that both proteins bind to Skp1 to form an SCF complex.

The yeast cells that overexpressed wild-type FLAG-Hrt3 or FLAG-Ylr224w were resistant to methylmercury, while yeast cells that overexpressed the proteins with deleted F-box domains (FLAG-Hrt3ΔF and FLAG-Ylr224wΔF) were not

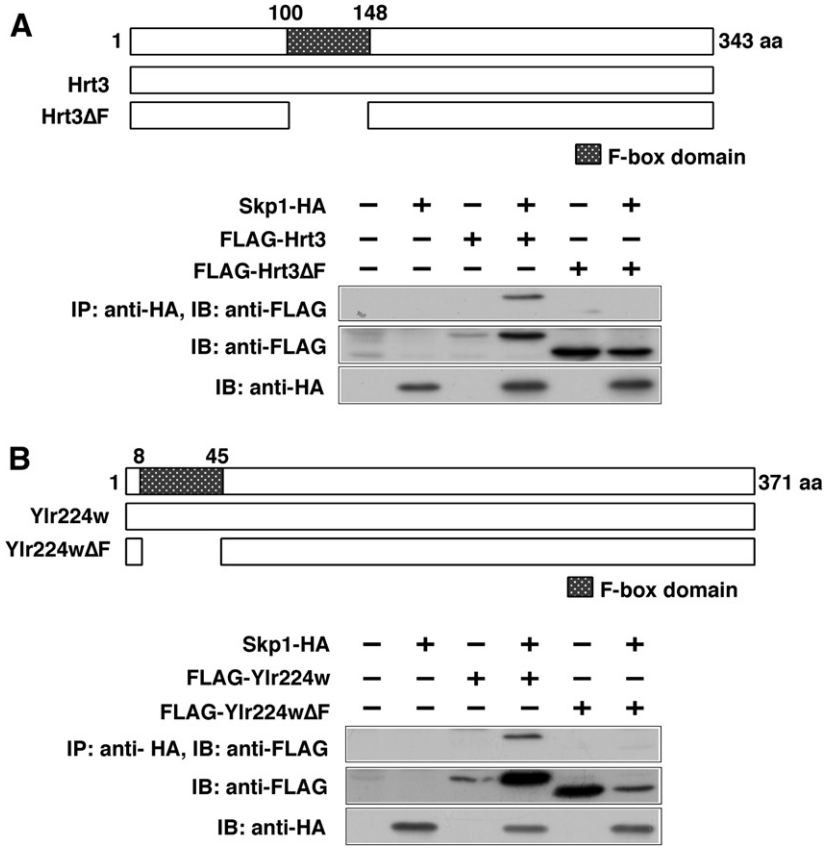

Fig. 2. Effects of deletion of the F-box domains of Hrt3 and Ylr224w on their binding to Skp1. A yeast strain expressing Skp1 fused to the HA tag (Skp1-HA) was transformed with pKT10, pKT10-FLAG-*HRT3* or pKT10-FLAG-*HRT3ΔF* (A) and pKT10, pKT10-FLAG-*YLR224W* or pKT10-FLAG-*YLR224wΔF* (B). Cell extracts were prepared from the various strains and analyzed by immunoprecipitation (IP) or directly by immunoblotting (IB). The Skp1-HA protein was immunoprecipitated from cell extracts with the monoclonal HA-specific affinity matrix (anti-HA). Immunoblotting analysis was performed with the FLAG-specific monoclonal antibodies (anti-FLAG).

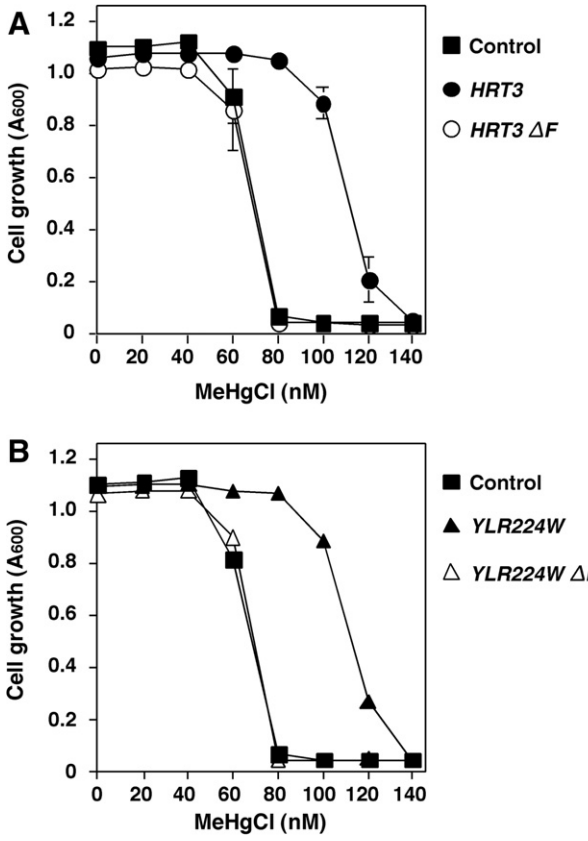

Fig. 3. Effects of deletion of the F-box domains of Hrt3 and Ylr224w on the sensitivity of yeast cells to methylmercury. Yeast cells (10^4 cells/200 μl/well) that harbored pKT10, pKT10-FLAG-*HRT3* or pKT10-FLAG-*HRT3ΔF* (A) or pKT10, pKT10-FLAG-*YLR224W* or pKT10-FLAG-*YLR224WΔ* (B) were grown in SD (−Ura) liquid medium that contained methylmercury at the indicated concentration. For other details, see legend to Fig. 1B.

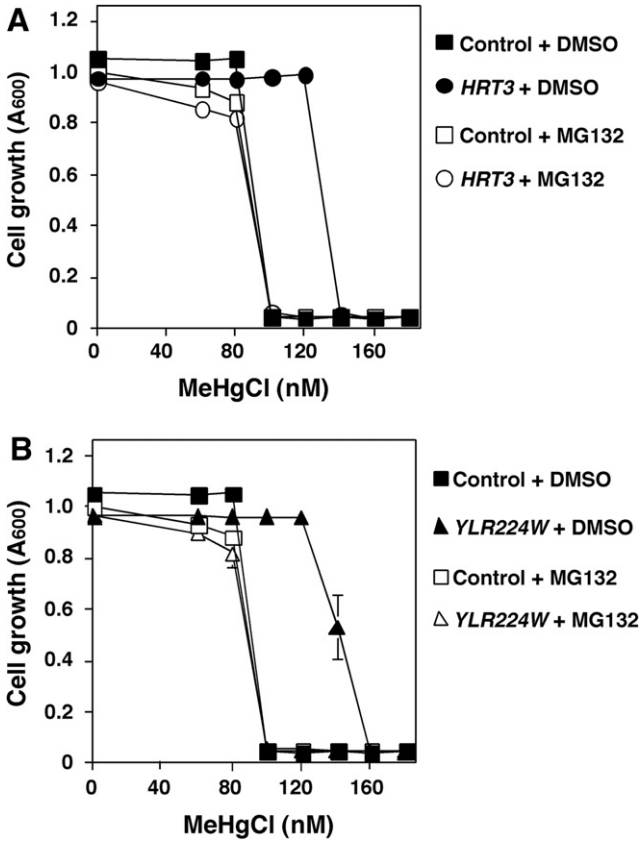

Fig. 4. Effects of a proteasome inhibitor on the Hrt3- and Ylr224w-mediated resistance of yeast cells to methylmercury. Yeast *erg6Δ* cells (10^4 cells/200 μl/well) that harbored pKT10 or pKT10-*HRT3* (A) or pKT10 or pKT10-*YLR224W* (B) were grown in SD (−Ura) liquid medium, with or without the proteasome inhibitor MG132 (50 μM), which had been dissolved in DMSO, and methylmercury at the indicated concentration. After incubation for 72 h at 30 °C, absorbance was measured spectrophotometrically at 600 nm. For other details, see legend to Fig. 1B.

(Fig. 3). Similar results were obtained when derivatives of both F-box proteins without FLAG tags were overexpressed in yeast cells (data not shown). Our results suggested that formation of an SCF complex might be necessary if yeast cells are to exhibit resistance to methylmercury upon overexpression of Hrt3 and Ylr224w and that both proteins act as F-box proteins in yeast cells.

Proteins that are polyubiquitinated via the ubiquitin system should be recognized and degraded by proteasomes. Therefore, we examined the possible role of proteasomes in the acquisition of resistance to methylmercury upon overexpression of Hrt3 and Ylr224w using MG132, a proteasome inhibitor [22]. Wild-type yeast cells are unable to incorporate MG132. Thus, we used an *ERG6*-deleted (*erg6Δ*) yeast strain whose cell membrane is more permeable than that of wild-type strains to MG132 [10,23]. Overexpression of both F-box proteins resulted in resistance to methylmercury in *ERG6*-deleted yeast cells, as anticipated. However, the resistance to methylmercury of *ERG6*-deleted yeast cells that overexpressed Hrt3 or Ylr224w disappeared in the presence of MG132 (Fig. 4). These results suggested that degradation of polyubiquitinated proteins by proteasomes was necessary for the resistance to methylmercury that resulted from the overexpression of the two F-box proteins. Therefore, a protein(s) that reinforces the toxicity of methylmercury might be included among those proteins that are recognized by Hrt3 or Ylr224w and are degraded by proteasomes after their polyubiquitination.

4. Discussion

The results obtained in this study demonstrate that Hrt3 and Ylr224w, two proteins with F-box domains, act as F-box proteins to reduce the toxicity of methylmercury through the formation of SCF complexes. Numerous proteins that are polyubiquitinated by the actions of E2 and the SCF complex are degraded by proteasomes. However, the transcriptional activity of Met4, for example, a transcription factor that is recognized and subsequently polyubiquitinated by the SCF complex that includes Met30 as its F-box protein, is suppressed upon its polyubiquitination, without subsequent degradation by proteasomes [24,25]. Therefore, polyubiquitination appears to be involved not only in protein degradation but also in the regulation of the activities of enzymes and other cellular factors. The present study revealed that overexpression of Hrt3 and of Ylr224w rendered yeast cells resistant to methylmercury, and resistance almost disappeared upon treatment of permeable yeast cells with the proteasome inhibitor MG132

(Fig. 4). Our results suggest that the resistance to methylmercury conferred on yeasts by overexpression of these two F-box proteins might be derived from enhanced degradation in proteasomes after enhanced polyubiquitination of a protein or proteins (hereafter collectively designated X-protein) that reinforce the toxicity of methylmercury.

In this study, disruption of the genes for Hrt3 and/or Ylr224w did not affect the sensitivity of yeast cells to methylmercury (data not shown). Thus, it is possible that X-protein might also be recognized and polyubiquitinated by some F-box protein(s) other than Hrt3 and Ylr224w.

Multiple E2s coexist with multiple F-box proteins in yeast cells. Therefore, it is possible that not only F-box proteins but also E2 might be involved in the selection of proteins as substrates for degradation. As described above, we found previously that overexpression of Cdc34, an E2, also rendered yeast cells resistant to methylmercury and, thus, the F-box protein related to this phenomenon might be Hrt3 or Ylr224w. However, when we overexpressed Cdc34 in yeast cells in which the Hrt3 and/or the Ylr224w gene had been deleted, the resistance of all three lines of cells to methylmercury was almost as strong as that of wild-type yeast cells that overexpressed Cdc34 (data not shown). This result suggests that Cdc34 does not confer resistance to methylmercury in cooperation only with SCF complexes that contain Hrt3 or Ylr224w as the F-box protein. However, we have also found that some types of E2 other than Cdc34 confer resistance to methylmercury when overexpressed in yeast cells [4]. Therefore, it is possible that types of E2 other than Cdc34 or multiple types of E2, including Cdc34, might play a role in the polyubiquitination of X-protein by SCF complexes that contain Hrt3 or Ylr224w as the F-box protein.

Their functions of Hrt3 and Ylr224w as F-box proteins were confirmed for the first time in the present study, but no further information is currently available, to our knowledge, about the possible proteins that might be polyubiquitinated after being recognized by these F-box proteins. In the preliminary study, we have already identified some candidates of X-proteins, which play a role in the enhancement of the toxicity of methylmercury and which are degraded by proteasomes after its polyubiquitination, by searching for proteins that can bind to both F-box proteins. The SCF complex, an E3 that includes F-box proteins, is present not only in yeast cells but also in mammalian cells, including human cells, and it has the same function in mammals as in yeast [26,27]. The identification of X-protein should help to clarify the mechanism of damage to the central nervous system that is caused by methylmercury.

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